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DNA Methylation in Oocytes and Liver of Female Mice and Their Offspring: Effects of High-Fat-Diet–Induced Obesity

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Running title: Obesity and DNA methylation in oocytes

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Abstract

Background: Maternal obesity has adverse effects on oocyte quality, embryo development and it also affects the health of the offspring.

Objectives: To understand the underlying mechanisms responsible for these negative effects, we investigated the DNA methylation status of several imprinted genes and metabolism-related genes.

Methods: A high-fat-diet (HFD)-induced mouse model was utilized to analyze the DNA methylation of several imprinted genes and of metabolism-related genes in oocytes from obese mice and in oocytes and liver from their offspring by employing combined bisulfite restriction analysis (COBRA) and bisulfite sequencing (BS).

Results: The DNA methylation of imprinted genes in oocytes was not altered in both obese mothers and their offspring, while DNA methylation of metabolism-related genes was changed. The DNA methylation level in the promoter of *Leptin* was significantly increased and that in the promoter of *Ppar-alpha* (*Ppar-α*) was reduced in oocytes of obese mice. The increased methylation of *Leptin* and decreased methylation of *Ppar-α* was also observed in the liver of female offspring from obese mothers (OHFD). The mRNA expressions of *Leptin* and *Ppar-α* were significantly altered in the liver of offspring from obese mothers. In OHFD oocytes, the DNA methylation level in the promoter of *Ppar-α* was increased.

Conclusions: These results indicate that DNA methylation patterns of several metabolism-related genes are not only changed in oocytes of obese mice, but also in oocytes and

liver of their offspring. These data may contribute to elucidating the adverse effects of maternal obesity on reproduction and the offspring's health.

Introduction

The World Health Organization reports show that obesity defined by abnormal or excessive fat accumulation that may impair health has nearly doubled since 1980 and there were nearly 300 million women being obese in 2008 (http://www.who.int/mediacentre/factsheets/fs311/en/). A number of years ago obesity and overweight was a problem in developed countries, but it has now become a problem in the entire world. Humans displaying obesity are prone to onset of type 2 diabetes, hypertension, cardiovascular disease and other disorders or diseases (Howie et al. 2009) and these conditions can be transmitted to the next generations (Fullston et al. 2012; Howie et al. 2009).

Obesity is a well-established cause of sub-fertility in humans and animals. When mice were fed a high-fat-diet (HFD) for 16 weeks, the ovulation rate, embryo development, placental function, ovarian function, and mitochondrial functions were affected in oocytes (Cardozo et al. 2011; Igosheva et al. 2010; Jungheim et al. 2010; Minge et al. 2008). The offspring of obese female mice showed a significant increase in body length (Dunn and Bale 2009). In humans, similar results were reported for oocytes of mothers with a higher BMI (body mass index), (Wattanakumtornkul et al. 2003) and their children tended to accumulate more fat by 9 years of age (Gale et al. 2007). These reports not only show that obesity causes female sub-fertility, but also that these adverse effects can be inherited by the offspring.

It is well known that obesity is not always caused by genetic mutation, but that the environment and life styles are key reasons as well. Currently, overweight and obesity are mainly attributed to lifestyle factors, such as excessive consumption of energy-rich food, low physical activities and other factors (McAllister et al. 2009). A number of studies have provided evidence that macro- or micro-nutrients induce epigenetic changes in offspring (Heijmans et al. 2008; E. W. Tobi et al. 2009; Waterland and Jirtle 2003; Waterland et al. 2006). Therefore, epigenetic alterations may be an important link between environment and genes by which obese parents transmit deleterious conditions to their children.

Genomic imprinting is a parental origin-specific gene-marking phenomenon which is crucial for normal mammalian development. Differentially Methylated Regions (DMRs) regulating expressions of imprinted genes are methylated on either the paternal or maternal allele (Reik et al. 2001; Sasaki and Matsui 2008). The DNA methylation status is established during the period of gametogenesis and early embryo development (Lucifero et al. 2002). However, methylation patterns of genomic imprinting genes tend to be altered by a deleterious environment or manipulation (Anckaert et al. 2010; Khosla et al. 2001). But the detailed mechanisms underlying these changes are still unknown.

Based on previous reports, we hypothesized that maternal obesity may impair DNA methylation of imprinted genes in oocytes and that it can be transmitted to the offspring. To test our hypothesis, we utilized HFD-induced obese mice, a widely used animal model (Igosheva et al. 2010; Jungheim et al. 2010; Minge et al. 2008), in our experiments. We investigated the

methylation patterns in DMRs of paternally imprinted gene *H19*, maternally imprinted genes *Peg3, Snrpn, Igf2r* and *Peg1* in oocytes of obese animals and their offspring. Because other studies had shown that the expression of *Leptin* and *Ppar-α* (peroxisome proliferators-activated receptor alpha) is regulated by DNA methylation in their promoters and the two genes are correlated to metabolism (Burdge et al. 2009; Cordero et al. 2011a; Cordero et al. 2011b) we also investigated DNA methylation levels of these two genes.

Materials and Methods

Mice provided by the Beijing Vital River Experimental Animals Centre were fed under conditions of light cycles of 12h light and 12h dark in a temperature (23 ± 1˚C) and humidity (60 ± 5%) controlled room. All procedures described were reviewed and approved by the Ethics Committee of the Institute of Zoology, Chinese Academy of Sciences. Mice were treated humanely and with regard for alleviation of suffering. In this study, we mainly analyzed the DNA methylation patterns of several genes in oocytes using a high-fat-diet-induced mouse model.

Obese mice

Weaned CD-1 mice, 3 per cage, were randomly divided into two groups. They were fed with high-fat-diet (HFD, D12492, Research Diets, America) or control diet (CD, see Supplemental Material, Table S1) for 12 weeks. We analyzed blood glucose concentration utilizing a

glucometer, Blood Testing Equipment, Accu-CHEK Active (Roche Diagnostic, Germany) (Ge et al. 2013).

Oocyte and liver collection

Mice were super-ovulated by a single intraperitoneal injection with 8IU pregnant mare serum gonadotropin (PMSG) and injection with 8IU human chorionic gonadotropin (hCG) (Tianjin Animal Hormone Factory, China) 46-48 hours (h) later (100 μl/mouse). After 13-14h, mice were sacrificed by cervical dislocation and MII (the second metaphase of meiosis) oocytes were collected from oviductal ampullae. Cumulus cells were removed using 1 mg/ml hyaluronidase (Vergara et al. 1997). Oocytes were washed in M2 medium (Sigma, USA) until no cumulus cells were observed in the medium; oocytes with attached cumulus cells were discarded. Oocytes were counted under the microscope. Oocytes of 7-8 week-old female offspring were collected as described above. The liver was collected at the same time.

Generation of offspring

The obese (n = 20) and control (n = 16) mice which had similar glucose level were mated with the same normal male mice. The time for observation of the vaginal plug in the morning was determined as gestational 0.5 day (d). The pregnant mice were housed in a single cage. Mothers were fed with HFD or CD during gestation and lactation. Offspring which were weaned at age of 21 days and housed 3 mice per cage after weaning in both groups were fed with control diet.

Bisulfite treatment and PCR amplification

Oocytes were subjected to bisulfite treatment and PCR analysis as we have previously reported (Ge et al. 2013). Briefly, protein K was added into tubes with 5 oocytes and incubated for 40 minutes (min) at 37˚C. Then the sample was denatured using 3M NaOH at 37˚C for 15min and modified by bisulfite solution (2.5 M sodium metabisulfite, Merck; 125 mM hydroquinone, Sigma; pH 5). Approximately 100 oocytes were used for each gene analysis in different groups.

DNA of livers was modified with EZ DNA Methylation-Direct™ Kit (ZYMO RESEARCH, USA) according to the manufacturer's instructions. Modified DNA was used as template of nested-PCR amplification. The relative primers were shown in Table S2 (see Supplemental Material, Table S2).

Combined bisulfite restriction analysis (COBRA) and bisulfite sequencing (BS)

COBRA and BS were carried out as recently reported by us (Ge et al. 2013). Briefly, we digested the product of PCR by one or two restriction endogenous enzymes. After digestion, the product of PCR was cloned to T vector and sequenced (Invotrigen, Beijing, China) according to the results of COBRA.

RNA purification and quantitative real-time PCR (qRT-PCR)

RNA was extracted from livers utilizing DNA Tissue Kit (Tiangen, China) according the manufacturer's instructions. The first cDNA strand was synthesized using Superscript II (Invitrogen). qRT-PCR was carried out utilizing Roche LightCycler480. Triple samples were analyzed for each gene and housekeeping gene of glyceraldehyde-3-phosphate dehydrogenase

(*GAPDH*) was used as a control. The expression level was evaluated by $2^{-\Delta\Delta Ct}$ (Ge et al. 2013). The primers were shown in Table S2 (see Supplemental Material, Table S2).

Statistical analysis

Data are represented as mean ± SD. The significance between groups was compared by independent-samples T test. The Chi-square test was used to evaluate whether significant difference exists in methylation density between different groups. A probability level of $P < 0.05$ was considered significant.

Results

Ovulated egg number and body weight in obese females and their offspring

The average body weight of female mice in the HFD group was significantly higher than that of the CD group (see Supplemental Material, Fig. S1A). After super-ovulation, the number of MII oocytes in obese females was significantly reduced (see Supplemental Material, Fig. S1B, $P < 0.01$) compared to CD mice. Although the number of MII oocytes in the offspring was similar between the two groups (see Supplemental Material, Fig. S1C), the average body weight of female and male was significantly higher ($P < 0.001$) for HFD offspring compared to that for CD offspring at 12 weeks of age (see Supplemental Material, Fig. S1D, E). The body weight of offspring from mothers fed with high-fat-diet (OHFD) at 12 weeks increased 19.4 ± 5.5% and 26.3 ± 8.4% ($P = 0.084$), respectively, for males and females compared to offspring of mothers fed with control diet (OCD).

DNA methylation patterns in DMRs of imprinted genes in mouse oocytes are not changed

Approximately 100 oocytes were used for each gene analysis in all groups. For *H19*, the bands digested by *Taq*$^{\alpha}$I and *Rsa*I showed that the DNA methylation in DMR was not affected by maternal obesity in oocytes (Fig. 1A). Similar results were obtained in DMRs of the maternally imprinted genes *Igf2r, Peg1,* and *Peg3* which were digested by *Taq*$^{\alpha}$I and *BstuI*, *Taq*$^{\alpha}$I and *BstBI*, *Taq*$^{\alpha}$I and *BstuI,* respectively (Fig. 1B-D). Although some samples of HFD oocytes were not completely digested by *BstuI* for *Snrpn*, similar bands were observed as in CD oocytes (Fig. 1E, shown by red arrowheads). Further bisulfite sequencing showed that the undigested bands of these samples were the result of DNA methylation changes at the loci of the recognition sites of *BstUI* (Fig. 2A, shown by red arrowheads). However, there was no significant difference between HFD and CD oocytes for *Snrpn* (Fig. 2A).

DNA methylation in promoters of *Leptin* and *Ppar-α* is altered in oocytes of obese females

For *Leptin*, the CpG island promoter was hypo-methylated in oocytes from CD mice (Fig. 2B). The methylation level was significantly higher ($P < 0.01$) in HFD mice compared to that of CD mice (Fig. 2B). For *Ppar-α*, we analyzed 14 CpG sites (Fig. 2C) which were marked 1 to 14 in the CpG island of the *Ppar-α* promoter. At sites 8 and 13 ($P = 0.13$), the methylation levels were slightly higher in oocytes from HFD mice compared to CD mice (Fig. 2D). But at the other sites, DNA methylation levels were obviously lower in HFD mice compared to those in CD mice. The

methylation of *Ppar-α* was significantly lower (P<0.01) in HFD mice compared to CD mice (Fig. 2D).

DNA methylation in the promoter of *Leptin* and *Ppar-α* is altered in liver of female offspring

For *Leptin* (Fig. 3A), the methylation level of the promoter in female liver was higher in offspring of the HFD group (OHFD, 81.2%, P = 0.013) compared to that in offspring of the CD group (OCD, 71.5%). For male offspring, the methylation level was also slightly higher (P = 0.138) in OHFD compared to that in OCD (Fig.3B). We evaluated the methylation level at promoter region of *Leptin* in liver between females and males. For females (71.5%), it was similar to that of males (72.0%, p = 0.898) in the OCD group. While the methylation level was slightly lower in males compared to that of females in the OHFD group (p = 0.179), but without significant difference.

At 2, 8, 13 and 14 CpG sites, the methylation level was decreased in female OHFD liver compared to female OCD liver (Fig. 3C) for *Ppar-α*. The mean methylation level in the promoter of *Ppar-α* was higher (P < 0.05) in female OCD than in female OHFD. In male offspring livers (Fig. 3D), the methylation patterns in promoter of *Ppar-α* were similar between OHFD group and OCD group (P = 0.877).

For both genes, the methylation patterns at CpG island in the promoter region control their expressions. We further investigated their expressions at the mRNA level and found that the expression level of *Leptin* in female liver of OHFD was significantly lower than that in OCD (p

$<$ 0.05, Fig. 3E). For *Ppar-α,* the expression in female liver of OHFD was clearly increased ($P < 0.05$, Fig. 3E) compared to that in OCD. But there was no significant difference for the expression of *Leptin* and *Ppar-α* in male liver of OHFD and OCD ($P = 0.275$ and 0.603, Fig. 3F).

DNA methylation patterns of imprinted genes in oocytes of OHFD are not altered

Approximately 100 oocytes were analyzed for each gene in both groups. The results showed that their methylation patterns were not altered in oocytes of OHFD (Fig. 4A-C). For *Snrpn*, there were some samples which were not completely digested by enzymes (Fig. 4D). Further analysis by BS showed that this was the result of DNA methylation changes at CpG loci located at the recognition site of *BstUI*. But the differences between the two groups were not significant (Fig. 5A).

The methylation levels of CpG sites in promoters of *Leptin* and *Ppar-α* in oocytes are different in OHFD and OCD

Approximately 100 oocytes were analyzed for each gene in both groups. We found that the methylation levels were very low in oocytes of both groups for *Leptin* (Fig.5B). Meanwhile, it was observed that the methylation levels at CpG sites of 6-10, 12, and 13 in the promoter of *Ppar-α* were increased in oocytes of OHFD compared to that of OCD (Fig. 5C). Similar methylation levels were detected in oocytes from OHFD and OCD at other CpG sites. The methylation level in the promoter of *Ppar-α* was significantly increased in the OHFD group compared to the OCD group (Fig. 5C, $P < 0.01$).

The methylation level of intracisternal A particle (IAP) elements is not altered in oocytes from obese mothers and their offspring

We evaluated the methylation status of IAP in oocytes of obese mothers and their offspring by BS. Approximately 100 oocytes were utilized in both groups. The results showed that the methylation level of IAP in oocytes was similar in the OHFD and the OCD groups (Fig. 5D). In oocytes of obese females, we found that the methylation pattern of IAP was not influenced, either (Fig. 5E).

Discussion

Establishing and maintaining proper DNA methylation is important for normal embryo development and the adult's health. DNA methylation modification links environment and gene expression. Previous studies have shown that malnutrition changed the DNA methylation status (Heijmans et al. 2008; Elmar W. Tobi et al. 2009; Waterland and Jirtle 2003). Our recent studies have revealed that postovulatory aging and maternal diabetes mellitus can alter DNA methylation patterns in DMRs of some imprinted genes in oocytes (Ge et al. 2013; Liang et al. 2008). Overweight and obesity mainly caused by eating excessively high-fat diet and by low levels of physical activity are among the largest worldwide threats to the health of our population (Finucane et al. 2011). Oocyte quality is decreased in obese mothers and that their children are predisposed to health problems (Igosheva et al. 2010; Jungheim et al. 2010; Minge et al. 2008). In this study, we found that obesity did not significantly affect DNA methylation in DMRs of

selected imprinted genes in oocytes, but it altered the DNA methylation levels of the promoters of *Leptin* and *Ppar-α* in oocytes.

Offspring of obese mothers are prone to having obesity problems as adults (Howie et al. 2009; Jungheim et al. 2010). Generally, there is no genetic mutation associated with this condition. Obesity is a metabolic disease and many studies (reviewed by Youngson and Morris, 2013) have demonstrated that it is related to changes in epigenetics (Youngson and Morris 2013). Therefore, in our study we investigated the DNA methylation levels of *Leptin* and *Ppar-α*, which are involved in metabolic processes and are regulated by DNA methylation (Cordero et al. 2011a; Lillycrop et al. 2008), in oocytes of obese females. We found that the methylation level in the promoter of *Leptin* was significantly increased in HFD group compared to CD group. The methylation level of *Leptin* in the liver of females was also increased in the OHFD group compared to the OCD group. This indicates that the abnormal DNA methylation status in the promoter of *Leptin* in oocytes may be maintained in the liver of OHFD. DNA methylation in its promoter controls the expression of *Leptin*, and if the *Leptin* expression level is lower, the individual tends to gain body weight (Allard et al. 2013; Cordero et al. 2011a). In the present study, we indeed found that the expression of *Leptin* in the female OHFD liver, corresponding to its higher methylation, was significantly decreased compared to the female OCD liver, while there was a slight decrease for male offspring ($P = 0.275$). This is consistent with the result that the average body weight of offspring from obese mothers is higher compared to that of offspring from normal mothers.

Ppar-α, as an isoform of PPARs, is a key factor for controlling systemic energy homeostasis, including adipocyte differentiation, inflammation and energy homeostasis, lipoprotein and glucose metabolism (Bensinger and Tontonoz 2008; Rakhshandehroo et al. 2010; Stienstra et al. 2007). We found that the mean methylation level in the promoter of *Ppar-α* was decreased in obese mouse oocytes, and in female OHFD liver its methylation level was still lower, especially at 2 and 14 CpG sites. Correspondingly, the expression of *Ppar-α* in female OHFD liver was obviously higher than that in female OCD liver ($P < 0.05$). Although we did not test the metabolism of glucose or lipid, offspring of obese mothers have lower glucose tolerance (Caluwaerts et al. 2007; Magliano et al. 2013). The expression of *Ppar-α* mRNA and protein in female OHFD liver was higher than that of the control (Zhang et al. 2005; Zhang et al. 2009). This coincides with our result on the expression of *Ppar-α* in liver. It has been reported that triglyceride levels are negatively correlated with the protein level of *Ppar-α* (Zhang et al. 2005) in liver of offspring from obese mothers. Park and Mun also report that mice fed with high-fat-diet have a higher *Ppar-α* level and reduced glucose tolerance in liver compared to the control (Park and Mun 2013). This is not contrary to the function of *Ppar-α* because many factors, such as CD36 and CPT-1 (Sato et al. 2002), participate in the process of regulating the expression of *Ppar-α* and glucose metabolism in liver (Fischer et al. 2003).

In the CD group, the DNA methylation level in the promoter of *Ppar-α* in oocytes was higher than that in the liver of OCD. A similar situation was observed in some CpG sites in the promoter of *Ppar-α* in obese mouse oocytes and in the liver of OHFD. These results indicate that

there may be a demethylation process in the promoter of *Ppar-α* during embryo development. The above results indicate that DNA methylation changes may play a key role in overweight or/and obesity of offspring in the high-fat-induced mouse model.

In animal models and humans, obese mothers have deleterious influences to the next generations (Dunn and Bale 2009; Gale et al. 2007). To demonstrate how obese mothers transmit the adverse effects to the next generations, we investigated the DNA imprinting in oocytes of OHFD mice. We found that DNA methylation patterns in DMRs of *H19, Peg3, Snrpn,* and *Igf2r* in oocytes were similar between OHFD and OCD groups. For *Leptin* or IAP, the methylation level was also not altered in OHFD oocytes. However, DNA methylation at CpG sites in the promoter of *Ppar-α* in oocytes was increased for OHFD compared to OCD (Fig. 5C).

The methylation status of *Ppar-α* was decreased in oocytes of obese female mice and in liver of female offspring from obese mothers, especially at the 2 and 14 CpG sites. This difference of methylation level in promoter region of *Ppar-α* between HFD oocytes, OHFD livers and OHFD oocytes may be induced by high-fat-diet during oocyte maturation and embryo development. During these processes, the DNA re-methylation and de-methylation are prone to being disturbed and this can be inherited by the next generations (Bergman and Cedar 2013; Seisenberger et al. 2013; Vrachnis et al. 2012). The adverse uterus environment and deleterious effects of the milk of obese mothers may also be reasons for the differences. But the detailed mechanism is still unknown and it is not clear whether this change could have effects on the next offspring generation.

Several studies have reported that expression of individual gene products in the human male and female placentae is different (Lehavi et al. 2005; Steier et al. 2004). When mice are fed with a low-fat diet or very high-fat diet, the female placenta displays more striking changes in gene expression compared to the male placenta at E12.5 and E15.5 (Gallou-Kabani et al. 2010; Mao et al. 2010). We found that the DNA methylation level in the promoter of *Leptin* and *Ppar-α* was significantly altered in the liver of female offspring from obese mothers, but in the male OHFD, there was no significant difference compared to OCD. Their expression coincided with the methylation pattern. Compared with OCD, the mean body weight at 12 weeks increased by 19.4 ± 5.5% and 26.3 ± 8.4% (P = 0.084), respectively, in male and female OHFD. The results of sexual dimorphism are consistent with previous findings. Many factors may play a role in this difference, such as blood flow from the maternal peripheral circulation to the utero-placental circulation, microRNAs, hormones, growth factors, placental structure and functions, and others (Clifton 2010). However, the detailed mechanism causing the differences in this study is still obscure.

In summary, DNA methylation is altered in oocytes, liver and oocytes of offspring in the high-fat-diet-induced mouse model, which may partly explain the adverse effects of obesity on reproduction and health in the offspring.

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Figure Legends

Figure 1. DNA methylation patterns in DMRs of imprinted genes in HFD and CD mice. DNA methylation in DMRs of paternally imprinted gene *H19* and maternally imprinted genes *Igf2r, Peg1, Peg3,* and *Snrpn* was analyzed in oocytes by COBRA. Ten mice were used for each gene analysis. Spermatozoa were used as a control. Enzymes used are shown in the right column. Control, CD; obesity, HFD; s, sperm; red arrowheads, undigested bands. (A) *H19,* sample of spermatozoa was digested and samples of oocytes were undigested; for (B) *Igf2r*, (C) *Peg1*, (D) *Peg3* and (E) *Snrpn,* sample of spermatozoa was undigested, but parts of oocyte samples were digested.

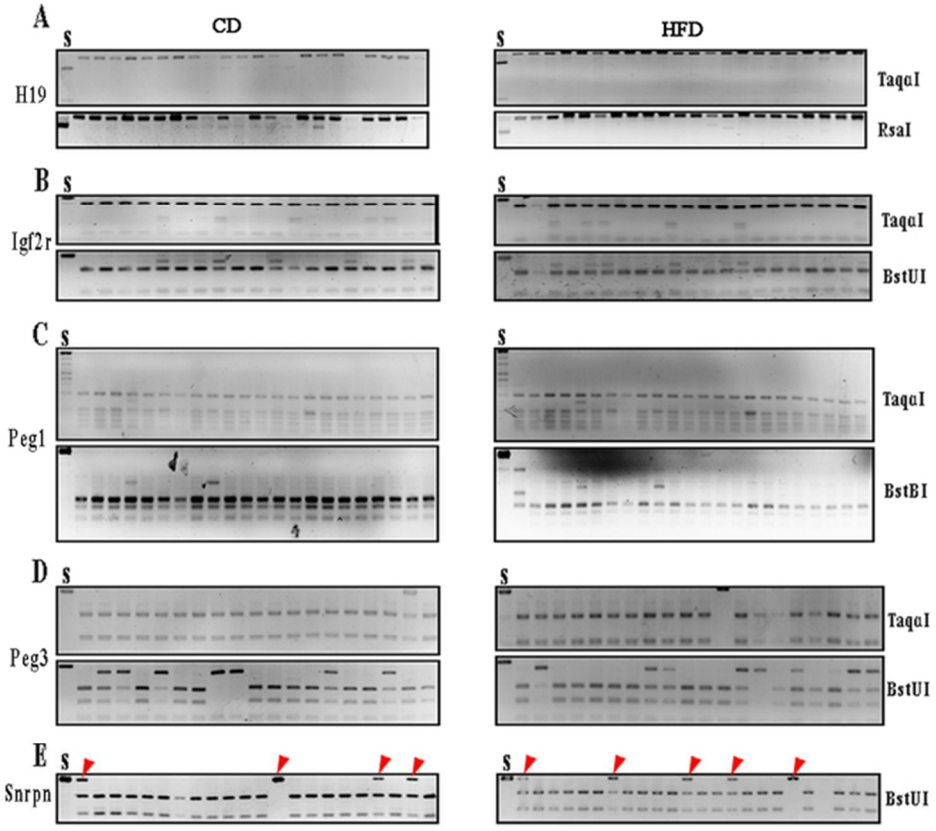
Figure 2. DNA methylation levels of *Snrpn, Leptin* and *Ppar-α* in oocytes of HFD and CD mice. DNA methylation of several genes was evaluated by BS. Ten mice were used for each gene analysis. (A) *Snrpn*, the red arrowheads show the recognition sites of BstUI; the number shows methylation %; (B) *Leptin* (analyzed region located at chr6: 26009934..26010283), number shows methylation %; (C) distribution of some CpG sites in promoter of *Ppar-α,* at which the methylation status was analyzed and CpG sites were marked by numbers from 1 to 14; (D) DNA methylation % at CpG sites in promoter of *Ppar-α*. Black bar, CD; white bar, HFD; black circle, methylated; white circle, unmethylated; blank loci, CpG lost.

Figure 3. *Leptin* and *Ppar-α* methylation and expressions in the offspring's liver. Livers were collected from females (n = 10 from 5 litters) and males (n = 10 from 5 litters) of OHFD

and OCD at age of 7-8 weeks, and DNA methylation was analyzed by BS. (A) DNA methylation of *Leptin* in liver of female offspring; (B) DNA methylation of *Leptin* in liver of male offspring; (C) DNA methylation at CpG sites of *Ppar-α* in liver of female offspring; (D) DNA methylation at CpG sites of *Ppar-α* in liver of male offspring; (E, F) expressions of *Leptin* and *Ppar-α* in female and male livers of offspring. Black bar, OCD; white bar, OHFD; black circle, methylated; white circle, unmethylated; blank loci, CpG lost; *P < 0.05.

Figure 4. DNA methylation patterns of imprinted genes in oocytes of offspring. DNA methylation in DMRs of paternally imprinted gene *H19* and maternally imprinted genes *Igf2r, Peg3,* and *Snrpn* was analyzed by COBRA in oocytes of OHFD and OCD. Ten mice from 5 litters were used for each gene analysis. Sperm sample was used as a control. Enzymes used are shown in the right column; S, sperm. (A) *H19*; (B) *Igf2r*; (C) *Peg3*; (D) *Snrpn*, red arrowheads show the undigested bands.

Figure 5. DNA methylation status of *Snrpn, Leptin, IAP,* and *Ppar-α* in oocytes of offspring. DNA methylation of these genes was analyzed by BS. Ten mice from 5 litters were used for each gene analysis. (A-B, D) representing methylation status of *Snrpn, Leptin* and *IAP* in offspring oocytes, respectively; the number shows methylation %; (C) representing DNA methylation at CpG sites of *Ppar-α*. Black bar, OCD; white bar, OHFD; (E) representing methylation patterns of IAP in CD and HFD oocytes, numbers show methylation %. ; black circle, methylated; white circle, unmethylated; blank loci, CpG lost.



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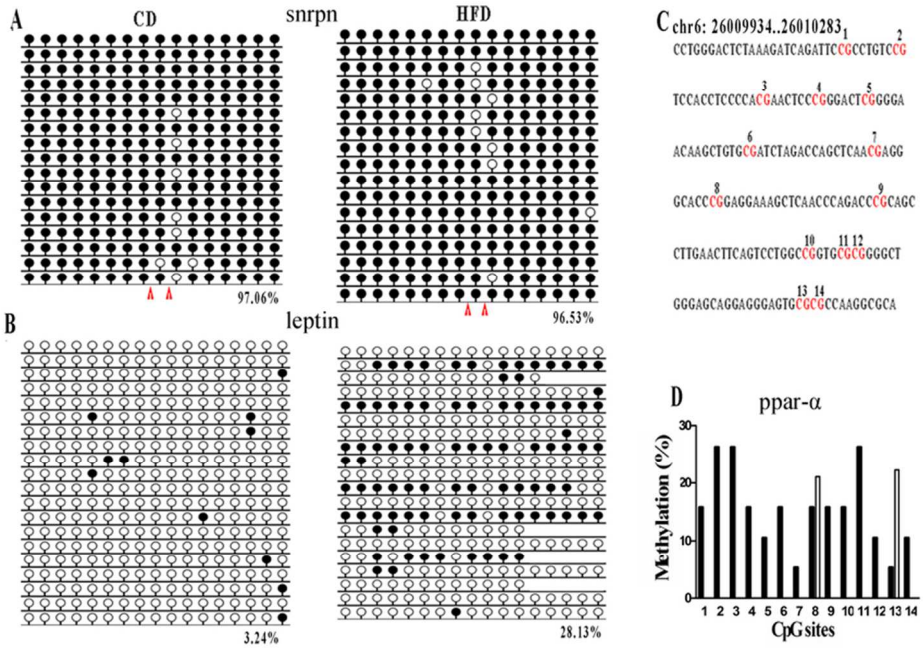

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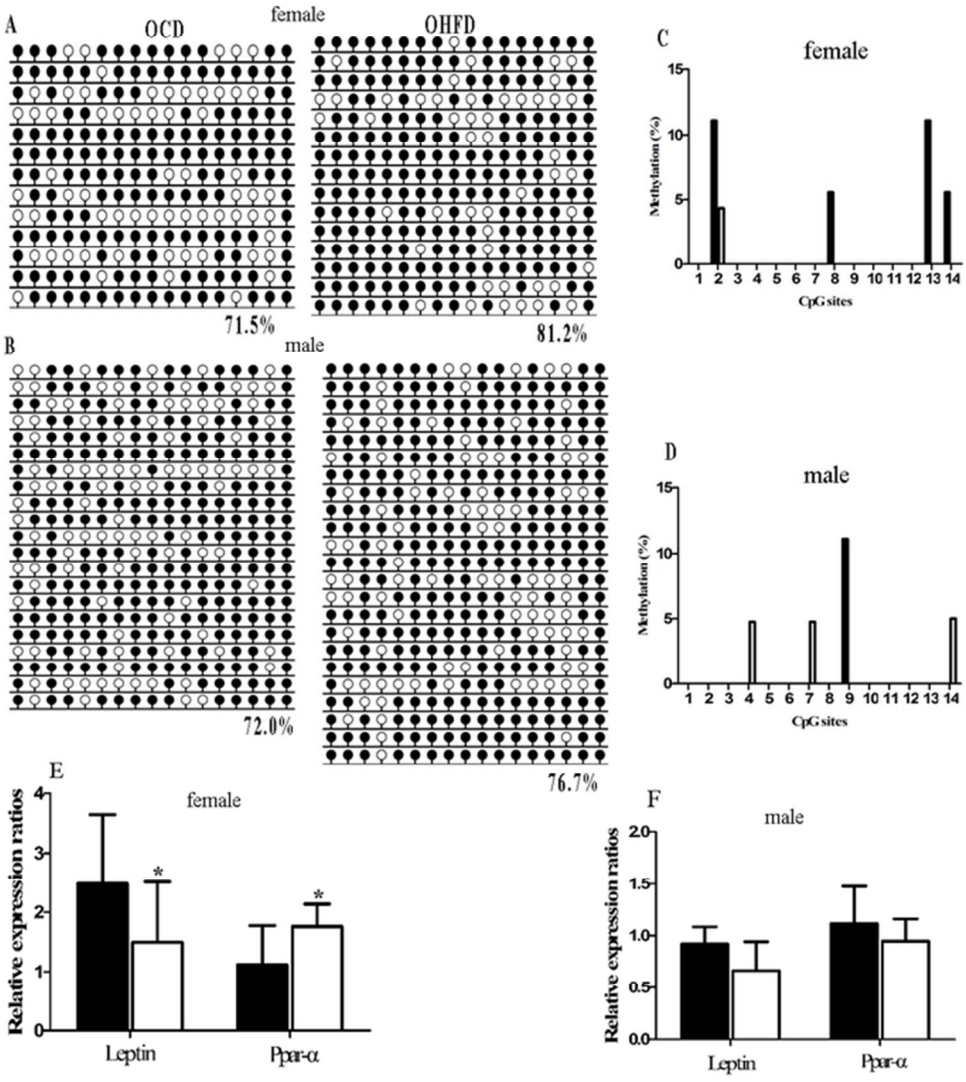

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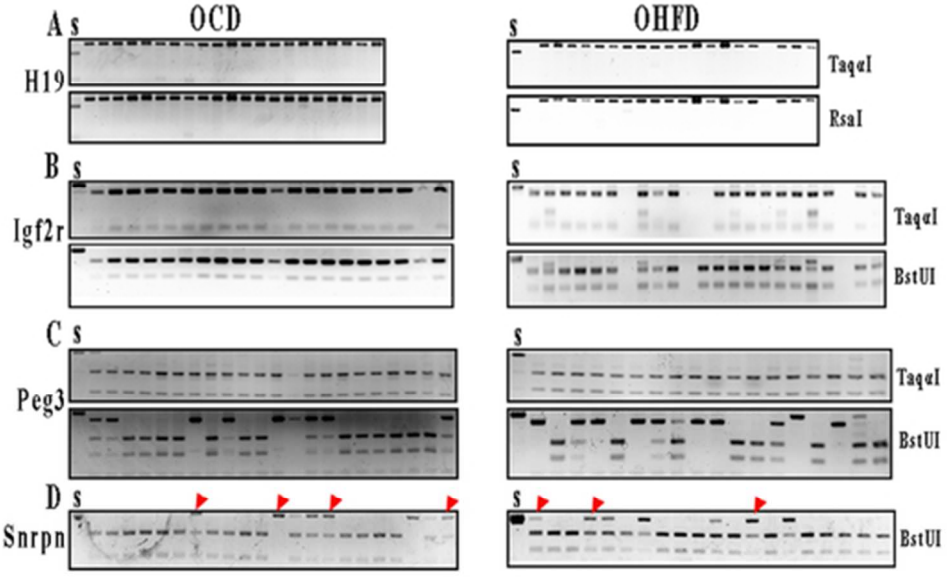

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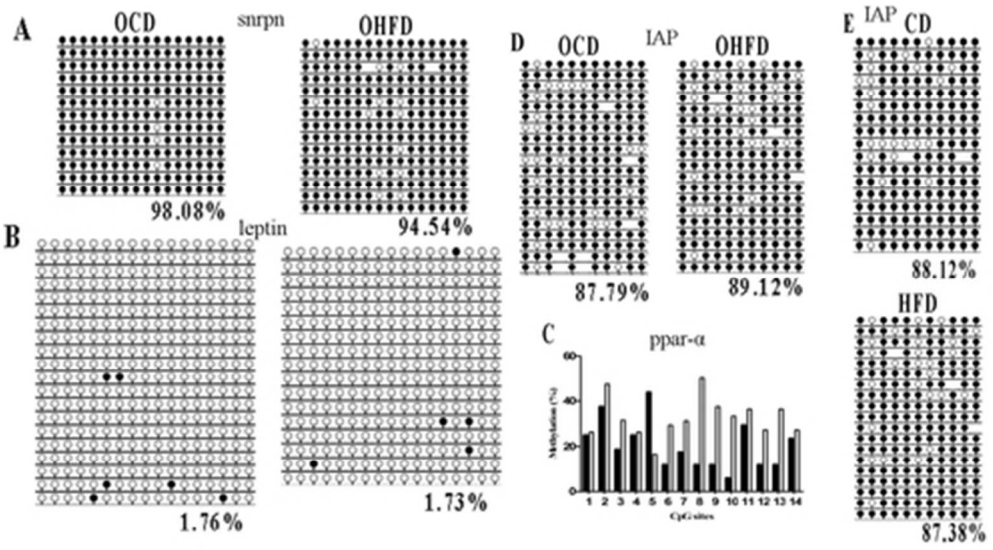

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